# Saskatchewan

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## FINAL REPORT

**INVESTIGATION OF THE ROLE OF BOVINE VIRUS DIARRHEA VIRUS AND MYCOPLASMA BOVIS IN CHRONIC PNEUMONIA OF FEEDLOT CATTLE**

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# Investigation of the role of bovine virus diarrhea virus and *Mycoplasma bovis* in chronic pneumonia in feedlot cattle

**Final report on project BDF041**
**to the Beef Cattle Development Fund,**
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The objectives of this study were to (a) define the features of a syndrome that has been recognized recently in feedlot cattle in western Canada in which cattle become chronic poor-doers with pneumonia and/or arthritis, and (b) to determine the role of bovine virus diarrhea virus (BVDV), *Mycoplasma bovis* and other agents in causing this disease.

The first step taken in the study was to review all cases with a history suggestive of this syndrome that had been submitted to the diagnostic pathology laboratory of the Department of Veterinary Pathology, WCVM, between January 1, 1995 and December 30, 1998. In total, the history, pathologic and microbiologic findings of 187 cases were reviewed. The history in these cases was generally that of a poor-doing animal that had been moved to a feedlot's chronic sickpen because of respiratory difficulty and/or lameness and that had failed to respond to antibiotic therapy. The lesions described at necropsy were usually of a severe chronic bronchopneumonia, sometimes with extensive fibrinous or fibrous pleuritis, and often with extensive, multifocal areas containing caseous necrotic material in the anterioventral portions of the lung. Fibrinous polyarthritis was sometimes present. The pneumonic and arthritic lesions in these feedlot cattle appear to be identical to those previously described in younger calves with fatal chronic pneumonia and arthritis associated with *M. bovis* infection (1,2). Based on review of these cases, there appears to be a distinct syndrome, the distinguishing features of which are chronicity, failure to respond to antibiotic therapy, severe bronchopneumonia with grossly visible areas of caseation in the anterio-ventral lung lobes, with or without concurrent fibrinous polyarthritis and tendosynovitis. In addition, many of these cattle had microscopic lesions, of unknown origin, present within blood vessels in the heart and/or lung, and occasionally in other tissues.

As the second step in the study, 48 of the above cases that had typical lung lesions, and from which suitable tissue samples were available in the WCVM files, were selected for further study. Multiple sections of heart and lung from each of animal were prepared and tested for the presence of the antigens of BVDV, *M. bovis* and *Haemophilus somnus*, using immunohistochemical staining. (*Haemophilus somnus* was included because it is involved in bovine respiratory and cardiac diseases, and has been associated with some forms of vascular injury).

In this retrospective study, *M. bovis*, BVDV, and *H. somnus* antigens were present in 94% (44/48), 64% (31/48) and 31% (15/48) of the animals, respectively. Microscopic damage to blood vessels was detected in the heart of 35 (73%) and lung of 26 (54%) of the animals. *Mycoplasma bovis* antigen was strongly associated with lung lesions and large amounts of antigen were present in and about the necrotic foci. In contrast, BVDV antigen was located predominantly in damaged areas of vessels in both heart and lung. *Haemophilus somnus* antigen was not associated with damaged vessels in any of the animals. Immunohistochemistry demonstrates the presence of antigen, but it is not possible to determine if live agents are present

using this technique. Complete microbiology had not been done on many of the retrospective cases; however, *M. bovis* had been isolated from eight of the nine animals that had been cultured specifically for mycoplasmas.

In order to obtain more complete information on the presence of viable pathogenic organisms in typical cases, a prospective study was performed. Sixteen new cases from among submissions to the diagnostic laboratory during 1999 were selected for study. The animals originated predominantly from two feedlots in Saskatchewan and had been submitted because of a history of chronic poor-doing and failure to respond to treatment. All of the animals had severe bronchopneumonia, typical of the syndrome. Five also had fibrinous polyarthritis and four had fibrinous tendosynovitis. Tissues were processed for both isolation of mycoplasmas, bacteria and virus, and for immunohistochemical staining for the same agents as in the retrospective study. *Mycoplasma bovis* antigen was detected in the lungs of 94% (15/16) of the animals and *M. bovis* was isolated from 13/16, either alone or in combination with other *Mycoplasma* spp. *Mycoplasma arginini* and *M. bovirhinis* were isolated the lungs of one case each. In the one case from which no mycoplasmas were isolated from the lung, *M. bovis* was recovered from a heart lesion. These results demonstrate clearly that viable mycoplasmas are extremely common in the lung lesions typical of this syndrome and persist in the tissues despite antibiotic therapy. Bovine virus diarrhea virus antigen was detected in 56% (9/16) of the animals, and BVDV was isolated from 4 animals. Two of the viruses isolated were identified as type Ib and the other two were type II. *Haemophilus somnus* antigen was detected in the heart of one animal and in the lung of eight animals. *Haemophilus somnus* was isolated from the lungs of three animals and a joint of a fourth animal. Injury to vessels was detected in the heart of 63% (10/16) and lungs of 56% (9/10) of the animals. Bovine virus diarrhea virus antigen was present at the site of vascular injury in 64% of these animals. *Haemophilus somnus* antigen was not detected in association with vascular injury in any animal.

The pneumonic and arthritic lesions in these feedlot animals appear to be identical to those described previously in younger calves (1,2) and the condition appears to be a syndrome with pathologic lesions distinguishable from other forms of respiratory disease in feedlot cattle. In young calves with pneumonia of this type, *M. bovis* has been considered to be the major pathogen responsible for lung injury (2). The presence of *M. bovis* in >90% of feedlot animals with typical lesions and the direct presence of this agent within the lung lesions supports the same conclusion in older cattle. Recognition of *M. bovis* as a respiratory pathogen in this age of cattle is important, because the agent is not susceptible to many antibiotics used for treating other forms of bovine respiratory disease, as was evident from the ability to isolate the agent from most of the animals in the prospective study. The exact role of BVDV in this and other diseases of feedlot cattle is not clear; however, the presence of viral antigen in >50% of the animals in this study and the association of the virus with vessel injury suggests that there is an association of this agent with the disease. None of the animals had lesions compatible with Mucosal Disease and we believe that the BVDV in these animals is a result of a primary rather than a persistent infection. Seroconversion to BVDV, indicating recent infection, has been reported to increase the risk of respiratory disease in feedlot cattle (3) and acutely infected cattle may undergo immunosuppression (4), predisposing them to other agents such as *M. bovis*. Vascular lesions have been reported previously in animals with Mucosal Disease (5) but, to our knowledge, these

have not been reported in primary BVDV infection or in association with respiratory disease. The presence of vascular lesions could be a valuable diagnostic indicator of exposure to BVDV in cattle. *Haemophilus somnus* may also play a role in the disease but was identified less frequently than the other agents and was not associated with the vascular injury.

Conclusions:

1. There is a distinct syndrome that occurs among feedlot cattle in western Canada that is characterized by chronicity, failure to respond to antibiotic therapy, severe bronchopneumonia with grossly visible areas of caseation in the anterio-ventral lung lobes, with or without concurrent fibrinous pleuritis, polyarthritis and tendosynovitis.

2. *Mycoplasma bovis* is present the lungs of these cattle and is strongly associated with areas of necrosis.

3. Viable *M. bovis* can be recovered from the lungs of these animals despite a history of antibiotic therapy.

4. A majority, i.e., >50%, of these animals have BVDV antigen in their tissues, and this antigen is strongly associated with microscopic damage to vessels.

5. *Haemophilus somnus* antigen was present in <50% of the animals and was not associated with vascular injury.

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This study formed the research component for a Master of Veterinary Science degree program in the Department of Veterinary Pathology, WCVM, completed successfully by Dr. Farshid Shahriar in June 2000. The results will be submitted for publication in the Canadian Veterinary Journal. A draft of that manuscript is appended and it contains details on the methods and techniques used. The Canadian Veterinary Journal was chosen as the publication most suitable for presenting this information to veterinary practitioners.

Thank you for support of this project.

G. Wobeser

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# Investigation of the role of bovine virus diarrhea virus and *Mycoplasma bovis* in chronic pneumonia of feedlot cattle

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**Abstract** - Chronic, antibiotic-resistant pneumonia, sometimes together with polyarthritis, occurs in cattle in feedlots in western Canada. Gross and microscopic lesions in these cattle are similar to those reported in *Mycoplasma bovis* infection in younger calves. We used histopathology, microbiology, and immunohistochemistry to determine the frequency of occurrence of *M. bovis*, bovine virus diarrhea virus (BVDV), and *Haemophilus somnus* in lung and heart of animals with this history submitted to the diagnostic pathology laboratory at the Western College of Veterinary Medicine, University of Saskatchewan. *Mycoplasma bovis* antigen was present in 91% (44/ 48) of retrospective cases submitted between 1995 and 1998, and 94% (15/16) of prospective cases from 1999, and was associated with necrotic areas within the lung. My*coplasma bovis* was isolated from 81% of prospective cases. Bovine virus diarrhea virus antigen was present in 64% (31/48) of retrospective and 56% (9/16) of prospective cases and was associated with lesions in vessels. Types Ib and II BVDV were each isolated from two of the 16 prospective cases. *Haemophilus somnus* antigen was present in the heart and lung of 31% (15/48) of retrospective and 50% (8/16) of prospective cases. Microscopic vascular lesions were present in the heart and lung of 77% (37/48) of retrospective and 69% (11/16) of prospective animals. Many of these vessels had positive staining for BVDV antigen; *H. somnus* antigen was not associated with vascular lesions. The presence of *M. bovis* and BVDV in a large proportion of cases suggests an association between these two agents in the pathogenesis of this syndrome of chronic pneumonia and arthritis of feedlot cattle

## Introduction

Bovine respiratory diseases are the most significant cause of mortality and elimination of calves from feedlots, with great economic impact on the cattle industry (1). Recently, multiple-source feedlots in the prairie region of western Canada have experienced many cases of antibiotic-resistant pneumonia, sometimes in association with fibrinous polyarthritis. The animals have chronic necrotizing bronchopneumonia, often with focal areas of caseation and bronchiectasis, and sometimes associated with fibrinous to fibrous pleuritis. They are eliminated from the herd because of lack of appropriate weight gain, poor doing and failure to respond to treatment. The major organism recovered from the pneumonia and/or arthritis is *Mycoplasma bovis,* either alone or in combination with other mycoplasmas including *M. arginini*, *M. bovirhinis*, and *M. bovigenitalium*. Lung lesions in these cases resemble those described in *M. bovis* infections in younger calves (2-6).

We have observed that these animals often have microscopic vascular lesions in the heart and lung, and occasionally in other tissues, which are positive in immunohistochemical staining for bovine virus diarrhea virus (BVDV). Other studies of *M. bovis* infection have not reported vascular lesions associated with BVDV. Several studies have shown that BVDV has a role in primary respiratory disease in calves (7, 8) and more severe pneumonic lesions have been described in infections of BVDV in combination with other respiratory pathogens (8-11). In one study, BVDV was recovered with high frequency from lungs of cattle (10). Impaired immunity in BVDV infected cattle has been suspected to be the main mechanism by which the virus affects the respiratory tract and,

hence, enhances the pathogenicity and virulence of other organisms (8). The immunosuppresive role of BVDV also has been suggested to result in increased pathogenecity of other microorganisms (5, 12).

We did a retrospective and prospective study, using histology and immunohistochemistry to determine the association, frequency of occurrence, and distribution of *M. bovis*, BVDV and *Haemophilus somnus* in the lung and heart of feedlot animals with chronic necrotizing bronchopneumonia with caseation and bronchiectasis, that was unresponsive to antibiotic treatment.

## Materials and Methods

### Retrospective study

A total of 187 cases with a final diagnosis of mycoplasma pneumonia that had been submitted to the diagnostic laboratory of the Department of Veterinary Pathology, Western College of Veterinary Medicine (WCVM), Saskatoon, Saskatchewan between 1 January 1995 and 30 December 1998 were reviewed. Cases were from Alberta and Saskatchewan feedlots. From these, 48 cases were selected for further study on the basis of availability of paraffin-embedded tissue sections, including heart and lung. Multiple sections of heart and lung, stained with hematoxylin and eosin (H&E), were examined histologically for the presence of pneumonic lesions in the lung and myocardial lesions in the heart, with careful attention to vascular lesions in both tissues. Warthin-Faulkner stain (13) was used occasionally to demonstrate bacteria in lung lesions. Other tissues were examined if present. Multiple heart and lung sections from each case, with or without specific lesions, were then submitted for immunohistochemical staining (IHC) using an

avidin-biotin immunoperoxidase technique (14) for BVDV, *H somnus* and *M. bovis* antigens. Duplicates of each block were tested for each antigen. In this method, after antigen retrieval with protease XIV (Sigma, St Louis, MO), the tissues were exposed to primary antibodies and incubated overnight at 4°C. A biotinylated goat anti-rabbit or horse antimouse (BVD stain) (Vector, Burlingame, CA) was used as a secondary antibody. Staining was completed using a peroxidase-labelled avidin-biotin complex (Vectastain, ABC Elite, Vector, Burlingame, CA). Diaminobenzidine was used as a chromogen substrate. The primary antibody used for detecting *Mycoplasma bovis* was a polyclonal rabbit antiserum (Dr. J. Tulley, NAIAD, Fredricton, MD) at dilutions of 1:1000 and 1:2000; for *H. somnus* a rabbit polyclonal antiserum (Veterinary Infectious Diseases Organization, Saskatoon, SK) diluted at 1:2000 and 1:4000 was used; and for BVDV monoclonal antibody 15C5 (Cornell University, Ithaca, NY) was used at dilutions of 1:100 and 1:200. Positive control sections from isolation-confirmed cases were tested concurrently with test tissues. Stained tissues were scored as either positive or negative for the presence of each antigen.

**Prospective study**

Prospective cases were selected from submissions for necropsy to the Prairie Diagnostic Services (PDS) at the WCVM during 1999. Most of the animals originated from two large feedlots near Saskatoon, SK, that are serviced by WCVM field service clinicians. The majority of the animals were from the "chronic pen" at the feedlots. The submissions consisted of either intact animals or portions including tongue, glottis and epiglottis, trachea, oesophagus, lungs, heart, and adjacent lymph nodes (the "pluck") and multiple pieces of ileum, spleen, and, occasionally, skin. A complete necropsy was performed on

the intact carcasses. Tissues were collected from 16 animals with lesions typical of mycoplasma pneumonia (2, 3), with or without joint lesions. Respiratory tracts were examined grossly for severity, distribution, and type of lesions. A minimum of eight portions of lungs, from both left and right sides and all lobes was collected, whether they were affected or not. The heart was examined for abnormalities and sections of left and right ventricular walls, interventricular septum, and right and left atrium were collected. The ileum from 11 animals was examined for lesions on the mucosa, serosa, and Peyer`s patches. At least two cross sections of ileum were collected. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6μm, stained with H&E and examined by light microscopy. Immunohistochemical staining of heart and lung tissues was as described for prospective cases, and also was applied to ileum, spleen, and skin (if available)

Three portions of fresh lung tissue from affected areas were submitted to the diagnostic bacteriology laboratory of PDS at the WCVM. In cases with other lesions, such as arthritis or myocarditis, tissues from these areas also were submitted for culture.. Culture for bacteria was based on the standard methods in laboratory, including inoculation on blood agar, chocolate agar, and MacConkey agar. Mycoplasma culture was done using a standard method (15). Mycoplasmas were identified using a fluorescent antibody (FA) method and antisera to *Mycoplasma bovis*, *M. arginini*, *M. bovirhinis*, and *M. bovigenitalium*.

Samples of lung, heart, and ileum were submitted to the PDS virology laboratory at the WCVM for FA test and virus isolation for BVDV. Virus isolation was done by inoculating tissue homogenates onto fetal bovine tracheal cells (FBTr). The cultures were

repassed onto Mardin Darby bovine kidney (MDBK) cells and FBTr cells. Presence of BVDV antigen was confirmed by FA staining on cell smears of second passage cultures, using a pool of monoclonal antibodies 15C5 and 20.10.6 (Cornell University, Ithaca, NY) raised against BVDV. Bovine virus diarrhea virus antigen was detected by FA in frozen sections of fresh tissue using the same pool of monoclonal antibodies. A polymerase chain reaction (PCR) method was performed on isolated BVD viruses to identify their genotype(16).

## Results

### Retrospective study

*Mycoplasma bovis* antigen was found in 91% (44/ 48) of the retrospective cases by IHC. Microscopic lesions in the lungs of the 48 animals were characterized by severe, necrotizing, suppurative bronchopneumonia with bronchiectasis, abscessation and occasional sequestration of portions of lung. The lesions were classified into three main stages: acute (early), subacute, and chronic (late) stages. In the acute or early stage, there was a small amount of eosinophilic exudate within the bronchioles and terminal airways with intact epithelial lining. The exudate within the lumen was composed primarily of degenerate neutrophils, macrophages and proteinaceous material, and this material stained positively for *M. bovis* antigen. Affected airways might or might not have peribroncholar infiltration of mononuclear cells. In the subacute stage, there was partial loss of bronchiolar epithelium associated with areas of hyperplasia in the mucosa of airways. The lumen was dilated and filled with large amounts of eosinophilic necrotic exudate. *Mycoplasma bovis* antigen was detected most commonly in the necrotic exudate

and at the periphery of the lumen. Unidentified bacteria also could be demonstrated in this area with Warthin- Faulkner stain. Affected airways were surrounded by a zone of lymphocytes and plasma cells or by large multifocal lymphoid aggregations. In the chronic stage, lung lesions were characterized by multifocal, well-circumscribed, variably sized and shaped, amorphous eosinophilic foci of coagulative necrosis surrounded by a thin zone of degenerate neutrophils, cellular debris, pyknotic nuclei and a wider area of mononuclear inflammatory cells, consisting predominantly of plasma cells and macrophages mixed with fibroblasts. Clusters of fine granular bacteria were evident at the centre and periphery of necrotic foci. In cases with prominent marginal fibrous tissue, multiple areas of hemorrhage and neovascularization were present within the fibrous tissue. In some cases, focal mineralization was present within the eosinophilic necrotic material. Chronic lung lesions had large quantities of *M. bovis* antigen, particularly at the margins of necrotic foci, in association with the zone of necrotic debris extending through the mononuclear cell layer. One or all stages of lesions could be seen in a single lung. Lung adjacent to the lesions described above was severely congested with a mixture of macrophages and neutrophils within the alveolar spaces. There was marked and diffuse dilatation of interlobular septa and lymphatics by fibrinous exudate .

Results of bacteriologic culturing were not available for all cases. *Mycoplasma bovis* was isolated from eight of nine animals that had been cultured specifically for mycoplasmas. *Mycoplasma arginini* and *M. bovigenitalium* were each isolated from a single animal.

Vascular lesions, that included necrotizing fibrinoid vasculitis in the tunica media and tunica intima (Fig. 1), lymphoplasmacytic perivasculitis and proliferative reparative changes in tunica media and intima were observed in the heart of 73% (35/48) and the

lung of 54% (26/48) of the animals. None of the damaged vessels had endothelial injury or thrombosis. Bovine virus diarrhea virus antigen was present in the heart of 26 (54%) and lungs of 21 (44%) of the 48 animals. In total, 31/48 animals (64%) had BVDV antigen one or both tissues. The antigen was located predominantly in the damaged areas of small to medium sized arteries. *Haemophilus somnus* antigen was present in the heart of 2 animals and in the lung of 13 other animals but was not associated with vascular lesions in any of the animals.

**Prospective study**

All animals had moderate to severe bronchopneumonia with fibrinous to fibrous pleuritis. The affected anterio-ventral areas of the lungs were markedly consolidated and characterized by variably sized (0.5-3 cm) cavities filled with caseous necrotic material throughout the parenchyma (Fig. 2). In one animal, numerous small caseous nodular masses also covered the pericardial sac and pleural surfaces of the lungs and thoracic wall. Five animals had moderate to severe fibrinonecrotizing polyarthritis involving stifle, tarsus (hock), carpus, shoulder joints and, in one case, the hip joint. Fibrinonecrotizing tenosynovitis was observed in four animals, mainly involving the long digital extensor tendon. One animal had a large area of myocardial necrosis and sequestration in the left ventricular papillary muscle. Other grossly visible lesions included focal necrotizing tracheitis in two animals, necrotizing laryngitis in two animals, and one animal had deep necrotizing dermatitis and panniculitis.

Microbiological findings are summarized in Table 1. *Mycoplasma bovis* antigen was detected in lung from 94% (15/16) of the animals. The staining pattern was similar to that described in the retrospective study. *Mycoplasma* spp. were cultured from lungs of 15/16

animals. From five, both *M. bovis* and *M. arginini* were recovered; from two *M. bovis*, *M. arginini*, and *M. bovirhinis* were isolated; from six only *M. bovis* was recovered; and only *M. arginini* or *M. bovirhinis* was isolated from one animal each. Mycoplasma spp., predominantly *M. bovis,* were recovered from four of five affected joints. No mycoplasmas were isolated from lung or joints of one animal with severe polyarthritis and a large focal area of myocardial necrosis; *M. bovis* was cultured from the area of myocardial sequestration and *H. somnus* was isolated from joints. There were no were no areas of caseation in the lung and *M. bovis* antigen was not detected in the lung of this animal. Lesions in the lungs of the 15 animals with positive mycoplasma culture were similar to those previously described in the retrospective cases. *Pasteurella multocida* was isolated from lungs of six animals: *H. somnus* was recovered from lungs of three animals and a joint of one animal.

Microscopic vascular lesions were found in the heart of ten (63%) and the lung of nine animals (56%). Two animals had severe, fibrinonecrotizing vasculitis in multiple organs, including heart, lung, spleen, kidney, liver, and lymph nodes. One animal had fibrinoid necrotizing vasculitis confined to the submucosa of the ileum, and five animals had lymphoplasmacytic myocarditis, in addition to the lung and heart vascular lesions. Vascular lesions were not detected in three animals. One animal had severe mineralization of medium and large arteries in the lung and spleen without any associated inflammation. Mild mineralization of the tunica media and tunica intima was seen in two cases with mild vascular injury. Depletion of lymphoid cells in Peyer`s patches was common in these animals. It was characterized by markedly shrunken Peyer`s patches with severe reduction to complete absence of lymphoid cells from Peyer`s patches and

presence of a few macrophages with lipofuscin pigment. Occasional areas of lymphoid regeneration were noticed.

Bovine virus diarrhea virus antigen was detected in tissues of 9/16 animals (56%). Of these, antigens was found in the heart of eight (50%) and lungs of six (38%) animals. Seven animals with vascular lesions had BVDV antigen in the wall of damaged vessels. Immunohistochemical staining of the ileum was positive for BVDV antigen in 5 of 11 cases (45%). Using the FA test, BVDV was detected in three animals. The virus was isolated from four animals, two of the isolates were identified by PCR as type Ib and two were type II BVDV.

*Haemophilus somnus* antigen was present in the heart of one and the lung of eight animals. The staining was located in the centre of the necrotic areas in the lungs and was not associated with vascular lesions. .

## Discussion

This study demonstrated that mycoplasma infection, particularly with *M. bovis*, is very common in chronic pneumonia and/or arthritis in feedlot cattle in western Canada, and that the organism frequently can be isolated from the chronic pneumonic lungs. *Mycoplasma bovis* antigen was detected by IHC in the lung of > 90% of the cattle in both studies. Pulmonary lesions were characterized by a necrotizing, suppurative bronchopneumonia with bronchiectasis and caseation necrosis. Fibrinous to fibrous pleuritis was a common finding in these animals. The lesions were similar to those described in younger calves infected with *M. bovis* (2-6). Our results suggest that *M. bovis* should be considered as a principle pathogen in chronic unresponsive pneumonia of

feedlot cattle. In a previous study, mycoplasmas were recovered from about 90% of feedlot cattle with fatal pneumonia (17) and *M. bovis* was the major isolate. In another study, mycoplasmas were isolated from almost 90% of lungs; *M. dispar*, *M. bovis*, *M. arginini*, *M. bovirhinis*, and ureaplasmas were isolated with different proportions (18). We did not isolate *M. dispar* or ureaplasma; however, these are fastidious and difficult to isolate under routine conditions.

In addition to the characteristic pneumonic lesions caused by *M. bovis*, a variety of other lesions from which mycoplasma were cultured or which were positive on IHC were observed. Some of these lesions have not been associated with pneumonia or *Mycoplasma spp.* infection. The lesions included focal necrotizing tracheitis, necrotizing laryngitis, deep necrotizing dermatitis and panniculitis, and necrotizing myocarditis with positive *M. bovis* culture and IHC.

Increased severity of *M. bovis* infection might be influenced by factors such as virulence of the mycoplasma strains, changes in immune status of the host by other agents, or stressors exacerbating the pathogenesity of *M. bovis*. *Mycoplasma bovis* may become pathogenic after tissue trauma or other physiological stress (19). Decreased host immune response with *M. bovis* infection also has been described (19, 20). While the exact role of BVDV in chronic diseases of feedlot animals is unknown, the presence of BVDV antigen in 64% of retrospective and 56% of prospective cases, and the isolation of the virus from four prospective cases, suggest that there may be synergism between the virus and *M. bovis*. The frequency of occurrence of persistent infection (PI) with BVDV in cattle entering a feedlot in western Canada was reported to be <0.1% (21), but these animals may provide a source of virus for acute infection of other animals. We believe

that many of the animals in our study had primary infections. The pattern of IHC staining in all but one of the cases was primarily in smooth muscle of small to medium-sized arteries. In the ileum, viral antigen was located in Peyer`s patches, with no evidence of antigen in the mucosal epithelium or glands, a pattern that has been seen in experimental primary infection (22). Seroconversion to BVDV has been reported to increase risk of respiratory disease in feedlot cattle (23). It has been suggested that acutely infected cattle undergo immunosuppression (24), that might predispose to secondary infection such as with *M. bovis*.

The vascular lesions seen in this study included severe fibrinoid necrotizing vasculitis, lymphoplasmacytic vasculitis and perivasculitis, and proliferative reparative changes in tunica intima and tunica media of arteries, and also occasionally in veins, and did not involve the endothelial lining of the vessels. We found BVDV antigen in association with vascular injury in 64% (31/48) of retrospective cases and 56% (9/16) of the prospective animals. Cardiac lesions in feedlot cattle usually are associated with *H. somnus* infection (25, 26) but we did not find *H. somnus* antigen in association with cardiac vascular lesions. Presence of vascular lesions in a significant number of the animals with positive IHC staining for BVDV suggests that these vascular lesions are associated with acute BVDV infection. Vascular lesions have been reported in cattle with mucosal disease (27, 28). To our knowledge, vascular lesions have not been reported in association with acute (primary) BVDV infection. These lesions could be used as a diagnostic indicator for the presence or previous existence of BVDV in the body. Among the tissues examined, vascular lesions were found more often in the heart than in other tissues. Collecting

multiple sections of heart for histology should be encouraged during post mortem examination of cattle suspected to have BVDV infection

In the current study, *H. somnus* antigen was present in 15 animals (31%) in retrospective study and 9 animals (56%) in prospective study. *Hemophilus somnus* was not a common bacterial isolate from affected lungs. The role of *H. somnus* in chronic pneumonia is unclear and it was not associated with vascular injuries. Antibacterial treatment in feedlot likely affected the isolation of this and other bacteria from the lungs.

In conclusion, *M. bovis* antigen was detected in more than 90% of two samples of feedlot cattle with chronic pneumonia. Bovine virus diarrhea virus antigen was demonstrated in 64% of retrospective cases and 56% of prospective cases. This indicated an association between the two microorganisms in chronic pneumonia of feedlot cattle. Immunosuppressive properties of BVDV in acutely infected animals may be a predisposing factor for increased pathogenicity of *M. bovis*.

**Acknowledgement**

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Table 1. Occurrence of lesions and microbiological findings in 16 cases of chronic pneumonia of feedlot cattle

| | **Animal #** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Arthritis | - | - | - | - | - | - | + | + | - | + | + | - | - | - | + | - |
| Vasculitis | H[a],L | H,L[b] | H,L | H,L | H,L | - | H,L | H | H,L | - | H | H,L | - | - | - | L |
| **Immunohistochemistry** | | | | | | | | | | | | | | | | |
| Mycoplasma | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + |
| BVDV | +[H] | - | - | - | H,L | H,L | - | H | H,L | - | H | H,L | H,L | - | - | L |
| *Hemophilus somnus* | - | - | L | L | L | - | H | - | L | L | - | - | L | - | - | + |
| **Isolation** | | | | | | | | | | | | | | | | |
| *Mycoplasma bovis* | + | + | + | + | - | + | + H | + | + | + | + | + | + | - | + | + |
| *Mycoplasma arginini* | - | - | - | - | + | + | - | - | + | + | + | + | + | - | - | - |
| *Mycoplasma bovirhinis* | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - |
| BVDV | - | - | - | - | + | + | - | + | - | - | - | - | - | - | - | + |
| *Hemophilus somnus* | - | - | + | - | - | - | + | - | - | - | + | - | + | - | + | - |

a- Heart b- Lung